SAND3.0-003/PCT/US

In the United States Patent and Trademark Office

Re: Sanders, Ira et al
SN:10/535,504
Filed: 05/18/2005
For: Treatment of Mammalian etc.

Group Art Unit: 1645
Examiner: Swartz, Rodney

Mail Stop Non Fee Amendments
Hon Commissioner for Patents
PO Box 1450
Alexandria VA 22313-1450

Declaration under 37 CFR 132

Ira Sanders declares and says:

1. He is the applicant and inventor of the above identified application.

2. He graduated from Cornell University, BA 1975, from Creighton University, School of Medicine, MD 1980, served an Internship in General Surgery, Rhode Island Hospital, Brown University School of Medicine, 1981, a Residency in Otolaryngology, Mount Sinai Hospital, 1982-4, a Research Fellowship, Department of Otolaryngology, Mount Sinai School of Medicine, 1984-6. He was appointed Assistant Professor, Department of Otolaryngology, Mount Sinai School of Medicine, 1987-1993 and Associate Professor, Department of Otolaryngology, Mount Sinai School of Medicine, 1993-2003. He became CEO, Piasek Biotech Inc, a company formed to commercialize tetanus toxin and botulinum toxin 2003-present.

3. He began research into the uses of botulinum toxin on or about 1989, before Allergan Inc even bought the original company that developed botulinum toxin as a drug, Oculinum Inc. He used botulinum toxin clinically in his practice to treat patients since about 1990. He was contracted by Allergan Inc to assay their new formulation of botulinum toxin on or around 1999.

4. He is the senior author of numerous publications on botulinum toxin. Abstracts of five of these papers are attached hereto and made part hereof as Appendix A.

5. In order to establish the efficacy of the methodology of the present application, *inter alia* he conducted a comparison experiment on a female subject with severe allergy to dog hair.

6. In 2004 he obtained botulinum serotype E neurotoxin from List Biologics Lab. Botulinum E was chosen because of its short duration of action (4-5 days versus months for botulinum A).

7. In order to penetrate the skin he made a liposome suspension of botulinum E: 11 mg of egg yolk phosphatidylcholine and 3.5 mg of stearylamine were dissolved in 5 ml of chloroform. The mixture was dried under a vacuum hood in a 50 ml round jar. After drying, 50 units of botulinum E in 5 ml of phosphate buffered saline were added. The jar was vortexed to mix components. The solution was then added to a gel filtration column and after passing through 1 ml aliquots were separately collected. The initial aliquots were clear and were presumably saline with excess botulinum toxin E. Approximately the 3rd through 5th aliquots came through very milky appearing and were presumed to be liposomes or micelles.

8. In order to easily prove that an active component was present 1 cc of the mixture was placed on his forearm in a marked area. The skin appeared to absorb the solution within 5 minutes. The next day he exercised to the point of sweating and place blotting paper on his forearms. The area where the solution was placed did not sweat and the blotting paper was dry.

9. The female test subject had an allergy to dog hair. This is most noticeable when she rubbed the thin inner forearm skin against the dog's fur. When she did that both forearms broke out with hives and diffuse redness and were very itchy.

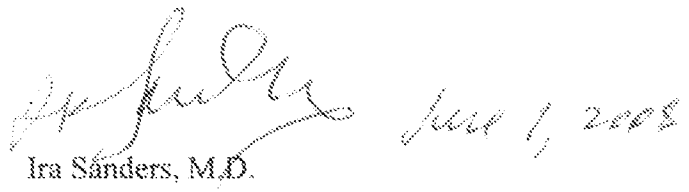
10. In the experiment, the botulinum solution was placed on her right forearm within the red dotted circle. When she rubbed against the dog her left arm broke out as usual but the area within the circle had no reaction, however, just below the circle an area of redness and hives can be seen. When the solution was made up without botulinum toxin it didn't give any protection. He repeated this experiment multiple times. The results are set forth in Exhibit A attached hereto and made a part hereof.

11. In Exhibit A:
    Top left. Both forearms showing where red circle is placed.
    Top right. Soon after contact with dog the left forearm turned red and hives began forming.
    Bottom left. No reaction within circle, although around it, such as lower down redness and hives appear.
    Bottom right. Left arm is very red and a clear rash is seen down the middle.

12. The results discussed in Paragraph 10 and Illustrated in Exhibit A, discussed in paragraph 11, clearly demonstrate the efficacy of botulinum toxin in countering the effects of allergic reaction.

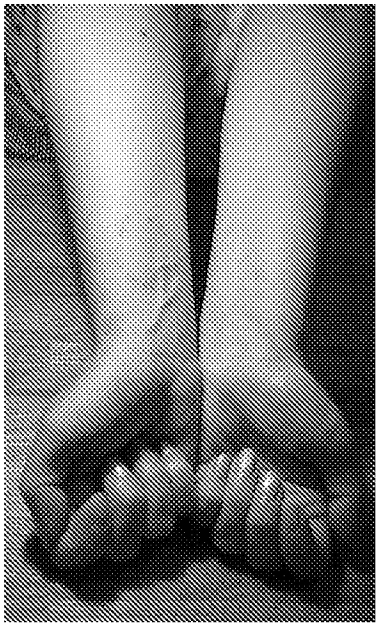
13. He hereby declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

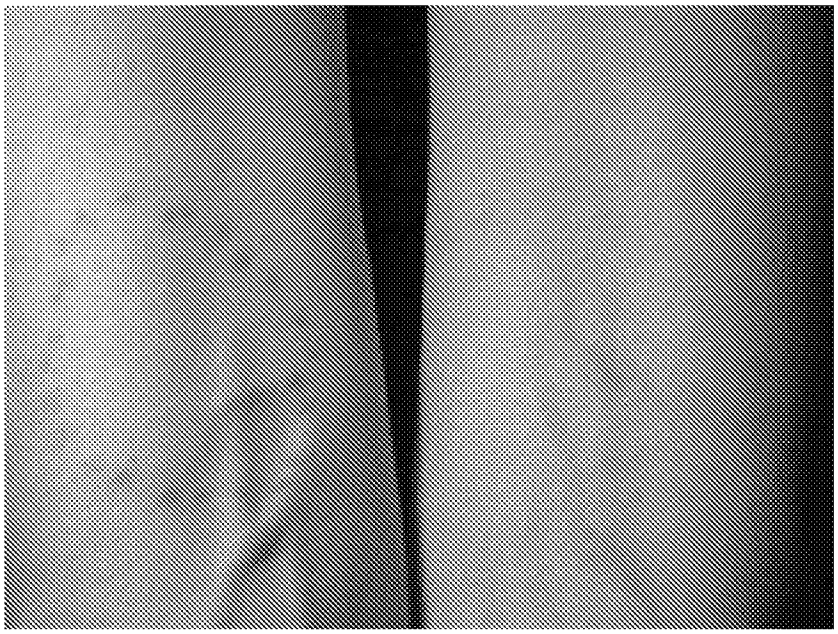
Further declarant saith not:

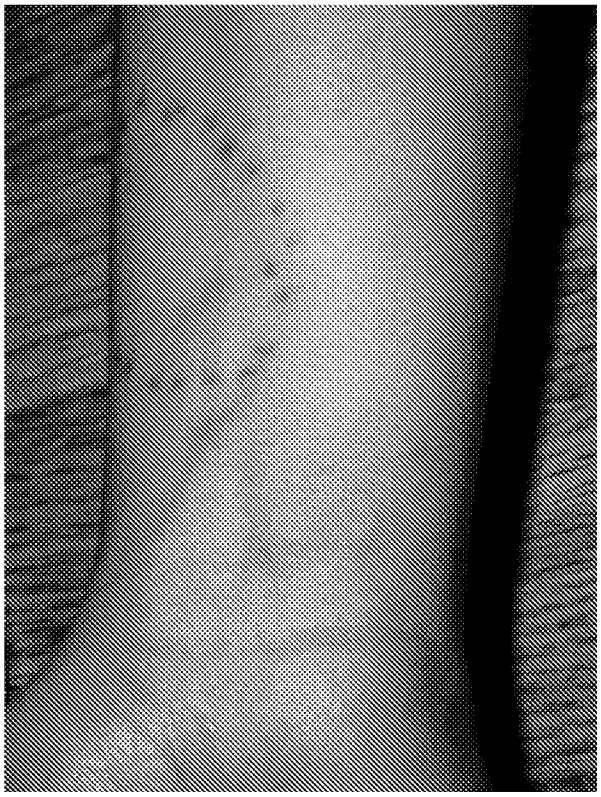


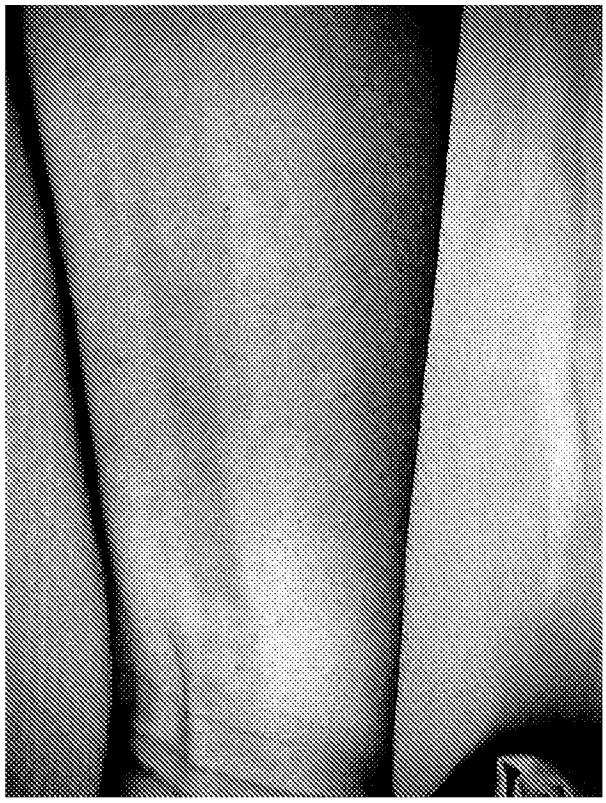
Ira Sanders, M.D.

EXHIBIT A









APPENDIX A

1: Otolaryngol Head Neck Surg. 1998 Apr;118(4):452-7.

Botulinum toxin decreases salivation from canine submandibular glands.
Shaari CM, Wu BL, Biller HF, Chuang SK, Sanders I.
Department of Otolaryngology-Head and Neck Surgery, Mount Sinai School of Medicine, New York, New York 10029, USA.

The objective of this study was to determine whether botulinum toxin types A and D reduced the production of saliva from the submandibular glands of 18 dogs. The left submandibular glands of 8 dogs were injected with increasing doses of botulinum type A toxin (range 10 to 70 units), and the left glands of 10 dogs were injected with botulinum type D toxin (50 or 100 units). The right gland of each dog was injected with equivalent volumes of saline solution to serve as control. Six days after the injection, the lingual nerve was electrically stimulated for 10 minutes (3 mAmp, 20 Hz). The resulting volume of saliva was collected and weighed. Overall, the glands injected with types A or D toxin produced significantly less saliva than comparable glands injected with saline solution. Six of 8 dogs injected with type A toxin showed a significant decrease in saliva production (range 10.1% to 19.2%, one-sided p value = 0.0375) when compared with the controls. Nine of 10 dogs injected with type D toxin demonstrated a highly significant reduction in saliva production (total average decrease = 60%, two-sided pvalue = 0.001) when compared with the controls. We concluded that intraglandular injections of botulinum toxin types A and D significantly reduced the production of saliva from canine submandibular glands. The potential applications of intraglandular injections of botulinum toxin are discussed.

Publication Types:
Comparative Study
PMID: 9560094 [PubMed - indexed for MEDLINE]

2: Otolaryngol Head Neck Surg. 1995 Apr;112(4):566-71.

Rhinorrhea is decreased in dogs after nasal application of botulinum toxin.
Shaari CM, Sanders I, Wu BL, Biller HF.
Department of Otolaryngology, Mount Sinai Medical Center, New York, NY 10029, USA.

At this time no effective long-term therapy exists for the excessive secretion of vasomotor rhinitis. Because rhinorrhea is under parasympathetic control, it was theorized that botulinum toxin--a powerful and long-acting cholinergic blocker that has been successful in the treatment of dystonia--might be useful in blocking the cholinergic control of rhinorrhea. Four male mongrel dogs were studied. Fifty units of type A botulinum toxin was soaked into sterile gauze, which was then packed into the left nasal cavity of each dog for 1 hour. Saline-soaked gauze was similarly introduced into the right nasal cavity to serve as control. Six days later, rhinorrhea was produced by inserting a bipolar needle electrode into the sphenopalatine ganglion and electrically stimulating for 10 minutes (6 mA, 50 Hz). Nasal secretions were collected with a suction catheter placed in the nasal vestibule. Three of four dogs exposed to the toxin showed a 41% average decrease in rhinorrhea (specifically 53%, 41%, and 30%). One dog showed a 10% increase in secretion after exposure to the toxin. We conclude that topically applied botulinum toxin reduced neurally evoked rhinorrhea by an average of 41%. Because some secretion is mediated by noncholinergic neurotransmitters such as vasoactive intestinal peptide, topical application of an anticholinergic substance has limitations. However, because all the nasal parasympathetic nerves

appear to originate from cholinergic synapses in the sphenopalatine ganglion, direct injections of toxin into this ganglion may possibly allow complete blockade of all cholinergically mediated rhinorrhea.

PMID: 7700663 [PubMed - indexed for MEDLINE]

3: Muscle Nerve. 1993 Sep;16(9):964-9.

Quantifying how location and dose of botulinum toxin injections affect muscle paralysis.
Shaari CM, Sanders I.
Department of Otolaryngology-Head and Neck Surgery, Mount Sinai Medical Center, New York, NY 10029-6574.

Despite the widespread use of botulinum toxin to treat muscle dystonias, no method exists to quantify muscle paralysis in either human or nonhuman models. In this study we examined how the location, dose, and volume of botulinum injection affects paralysis in the rat tibialis anterior muscle. Paralysis was quantified by electrically stimulating the nerve to the tibialis anterior and then staining sections of the muscle for glycogen. The areas of glycogen-containing fibers represented regions of botulinum action. The results showed that the most important injection technique is to inject botulinum directly into the motor endplate region of a muscle. Injections only 0.5 cm from the motor endplate resulted in a 50% decrease in paralysis. Increases in dose increased paralysis, however, some of that increase was simply due to the increased volume of injection. Thus, delivering toxin in small volumes near the MEP band of a muscle should produce the most effective paralysis.

PMID: 8355728 [PubMed - indexed for MEDLINE]

4: Ann Otol Rhinol Laryngol. 1992 Nov;101(11):888-92.

Quantitative mapping of the effect of botulinum toxin injections in the thyroarytenoid muscle.
George EF, Zimbler M, Wu BL, Biller HF, Sanders I.
Grabscheid Voice Center, Mount Sinai Medical Center, New York, New York 10029.

Spasmodic dysphonia has been successfully treated by thyroarytenoid muscle injections of botulinum toxin (Botox) with dosages ranging from 0.625 to 25 U. In some patients, excessive paralysis with resulting breathiness and aspiration have been noted. In order to maximize the efficiency of Botox injections, the histologic effects of various Botox dosages were examined in the dog. Nine canine thyroarytenoid muscles were injected with 0.5 to 12.5 U of Botox. After 24 hours, the recurrent laryngeal nerve to the injected muscle was electrically stimulated in order to deplete the glycogen within the muscle fibers. Frozen sections of this muscle were then stained for glycogen. Those fibers that retained their glycogen were presumed paralyzed by the Botox injection. The extent of paralysis was found to be dose-related from 1.0 to 7.5 U. At 10 U and above the muscle was completely paralyzed. Spread of the toxin to the lateral cricoarytenoid muscle was seen at doses as low as 1.0 U. Clearly, doses less than 10 U appear sufficient for clinical paralysis.

Publication Types:

Research Support, Non-U.S. Gov't

PMID: 1444095 [PubMed - indexed for MEDLINE]

5: Laryngoscope. 1991 Sep;101(9):960-4.
Quantifying the spread of botulinum toxin through muscle fascia.

Shaari CM, George E, Wu BL, Biller HF, Sanders I.
Department of Otolaryngology, Mount Sinai Medical Center, New York, NY 10029-6574.

Botulinum toxin was recently approved for treating several head and neck dystonias. Paralysis of neighboring muscles is the major complication of its use. Spread of toxin from the injected muscle has been suggested as an etiology. This study examines how botulinum toxin crosses muscle fascia by a novel method of quantifying muscular paralysis. Botulinum toxin (0.2 to 10 U) was placed onto the fascia of rat tibialis anterior (TA) muscles (n = 6). Toxin was also placed on dose-matched muscles that had their fascia surgically removed (n = 6). Twenty-four hours later, the nerve to the tibialis anterior was electrically stimulated to deplete the muscle fibers of glycogen. Toxin-paralyzed fibers retained their glycogen and appeared purple on periodic acid-Schiff (PAS) stain. Botulinum toxin easily passed through muscle fascia even at subclinical doses. The presence of fascia reduced the spread of botulinum toxin by 23%. These results suggest that spread of botulinum toxin can be prevented only by delivering small doses to the center of a target muscle.

Publication Types:

Research Support, Non-U.S. Gov't

PMID: 1886444 [PubMed - indexed for MEDLINE]